27. The method of claim 21, wherein the testing of the separated fractions of the first, second and third samples each comprise two determinations of the proteolytic activity, one carried out in the absence of and the other in the presence of a proteasome inhibitor.

REMARKS

Claims 15-20 are hereby cancelled without prejudice or disclaimer. New claims 21-27 are hereby added. Applicants respectfully submit that no new matter has been introduced by these amendments.

Claims 15-20 were rejected under 37 C.F.R. 112, second paragraph.

New claims 21-27 recite the subject matter of these now cancelled claims. Applicant respectfully submits that these changes overcome this rejection. Additionally, claims 16-21 formerly recited uses for the method of claim 15. Now, the counterparts of these claims, new claims 22-27, recite methods.

Claims 16-20 were rejected under 37 C.F.R. 112, first paragraph.

The present invention relates to the collection of crystallographic structural data and comparison of the new structures with known structures of proteasomes. The invention allows identification of new proteasome structures, either unobtainable or unsatisfactorily so by prior art methods. Generation of some crystal structural data is described in detail and in a reproducible manner in the Examples and the attached Figures found in Applicants' specification. Since the data is known in the art, the specific crystallographic data was not included in the specification. However, one of ordinary skill in the art can obtain the data from the Brookhaven Protein Database,

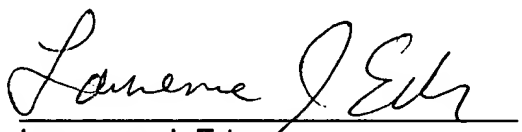
Accession No. 1RYP. A cover sheet of this pertinent entry is filed with this Response for illustrative purposes.

Applicants' claims now particularly point out and distinctly claim what Applicants' regard as their invention in the manner patently distinguished over all grounds of rejection cited in the office action. Accordingly, allowance of all claims is respectfully requested.

Please charge any fee deficiency or credit any overpayment to Deposit Account No. 01-2300.

Should the Examiner deem that any further action by the Applicants would be desirable for placing this application in even better condition for issue, the Examiner is requested to telephone applicants' undersigned representative at the number listed below.

Respectfully submitted,



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MARKED-UP VERSION OF ORIGINAL CLAIMS

21. (New) A method for identifying and isolating new proteasome inhibitors comprising:

obtaining eukaryotic cells;

lysing the cells, thereby producing a crude extract;

removing insoluble components from the crude extract, thereby producing a refined extract;

dividing the refined extract into first, second, and third extract samples;

separating the first sample into fractions by chromatographic separation with an ion exchange medium;

testing the separated fractions of the first sample to isolate the active fractions of the first sample;

separating the second sample into fractions by chromatographic separation over hydroxyapatite;

testing the separated fractions of the second sample to isolate the active fractions of the second sample;

pooling and concentrating the active fractions of the first and second samples;

separating the third sample into fractions by chromatographic separation over a gel filtration medium;

testing the separated fractions of the third sample to isolate the active fractions of the third sample;

crystallizing the pooled and concentrated active fractions of the first and second sample and the active fractions of the third sample;

analyzing the structure of the crystallized fractions, thereby identifying and isolating new proteasome inhibitors.

22. (New) The method of claim 21, wherein analyzing the structure of the crystallized fractions further comprises collecting data which is compared to known proteasomes, thereby identifying and isolating new proteasome inhibitors.

23. (New) The method of claim 21, wherein analyzing the structure of the crystallized fractions further comprises comparing the crystal structural data from the region of the proteasome pockets S1 of the subunits β1/PRE2, β2/PUP2 and β5/PRE2 to the crystallized fractions, thereby identifying and isolating new proteasome inhibitors.

24. (New) The method of claim 21, wherein analyzing the structure of the crystallized fractions further comprises collecting crystal structural data from the crystallized fractions, and processing that data with a computer-aided modeling program thereby identifying and isolating new proteasome inhibitors.

25. (New) The method of claim 23, wherein the computer-aided modeling program modifies the crystal structural data of a yeast proteasome with amino acid sequences from the human proteasome.

26. (New) The method of claim 21, wherein the three-dimensional structure of the crystallized fractions is complementary to the proteasome pockets S1 of the subunits β1/PRE2, β2/PUP2 and β5/PRE2.

27. The method of claim 21, wherein the testing of the separated fractions of the first, second and third samples each comprises two determinations of the proteolytic activity, one of which is carried out in the absence and the other in the presence of a proteasome inhibitor.

PDB
PROTEIN DATA BANK

Structure Explorer - 1RYP

Summary Information

Title: **Crystal Structure Of The 20S Proteasome From Yeast At 2.4 Angstroms Resolution**

Compound: **Mol_Id: 1; Molecule: 20S Proteasome; Chain: A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, 1, 2; Ec: 3.4.99.46; Mutation: Chains H, V, T1A, Chain L, Z, K33R; Biological_Unit: Yeast Proteasome Seems To Be Composed Of 14 Different Subunits Which Form A Highly Ordered Ring-Shaped Structure**

Authors: **M. Groll, L. Ditzel, J. Loewe, D. Stock, M. Bochtler, H. D. Bartunik, R. Huber**

Exp. Method: **X-ray Diffraction**

Classification: **Multicatalytic Proteinase**

EC Number: **3.4.99.46**

Source: **Saccharomyces Cerevisiae**

Primary Citation: **Groll, M., Ditzel, L., Lowe, J., Stock, D., Bochtler, M., Bartunik, H. D., Huber, R.: Structure of 20S proteasome from yeast at 2.4 A resolution.** ***Nature*** **386** ***pp.*** **463 (1997)**
[Medline]

Deposition Date: **26-Feb-1997** *Release Date:* **15-Apr-1998**

Resolution [Å]: **1.90** *R-Value:* **0.286**

Space Group: **P 1 21 1**

Unit Cell: *dim [Å]:* *a* **135.49** *b* **300.70** *c* **144.42**
angles [°]: *alpha* **90.00** *beta* **112.89** *gamma* **90.00**

Polymer Chains: **A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, 1, 2** *Residues:* **6386**

Atoms: **52604**

HET groups:

ID	*Name*	*Formula*
MG	MAGNESIUM ION	MG_1